Colony PCR

MARDI 17/08/2021

Preparing primers:

180μL RNA free water + 20μL of primer stock (->10 fold dilution)

Setting up the reactions:

- Collect a single colony into 50 ul of RNAse free water and lyse at 95°C for 2-5 minutes.
- Set up the reactions like so:

Table1

	A	B
1	Reagent	Volume
2		1x
3	Nuclease-free water	19 ul
4	2x DreamTaq Master Mix	25 ul
5	Forward Primer (10 uM)	2.5 ul
6	Reverse Primer (10 uM)	2.5 ul
7	Template	1 ul
8	Total Volume	50 ul

A master mix with the all components except for the template can be made to limit pipetting.
Vortex on very low to not damage the enzymes

- Set up the thermal cycler like so:

Table2

	A	B	C	D	E
1	Thermocycler Protocol: DreamTaq Master Mix				
2		Temp	Time		
3	Start	95 °C	1 min	Melt	
4					
5	Cycle 1	95 °C	30 sec	Melt	35 Cycles
6	Cycle 2	depends on annealing temps of primers*	30 sec	Anneal	
7	Cycle 3	72 °C	1 min	Extend	
8					
9	Finish	72 °C	5 min	Extend	
10	Store	10 °C	Forever	Store	

*55°C is a standard temperature